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Multilayered tension wood cell walls in Salicaceae

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Abstract

The circumscription of Salicaceae has recently been enlarged to include a majority of the species formerly placed in the polyphyletic tropical family Flacourtiaceae. Several studies have reported a peculiar and infrequently formed multilayered structure of tension wood in four of the tropical genera. Tension wood is a tissue produced by trees to restore their vertical orientation, and most studies have focused on trees developing tension wood by means of cellulose-rich, gelatinous fibres, as is known in *Populus* L. and *Salix* L. (Salicaceae *s.s.*). This study aims to determine if the multilayered structure of tension wood is an anatomical characteristic common in other Salicaceae, and if so, how its distribution correlates to phylogenetic relationships. Therefore, we studied the tension wood of 14 genera of Salicaceae as well as two genera of Achariaceae, one genus of Goupiaceae, and one genus of Lacistemataceae, families closely related to Salicaceae or formerly placed in Flacourtiaceae. Opposite wood and tension wood were compared with light microscopy and 3D Laser Scanning Confocal Microscopy. The results indicate that a multilayered structure of tension wood is common in the family except in *Salix*, *Populus*, and one of their closest relatives, *Idesia polycarpa* Maxim. We suggest that tension wood may be a useful anatomical character in understanding phylogenetic relationships in Salicaceae. Further investigation is still needed on the tension wood of several other putatively close relatives of *Salix* and *Populus*, in particular *Bennettiodendron* Merr., *Macrohasseltia* L.O.Williams and *Itoa* Hemsl.

Keywords

Flacourtiaceae – G-layer – Multilayered tension wood – reaction wood – Salicaceae

Introduction

Until recently, morphological and anatomical characters were the primary sources of data for inferring phylogenetic relationships. More recently, DNA data have provided the majority of characters for our analyses of relationships, but reference to morphological characters remains useful for many reasons, from pedagogy to comparative evolutionary studies to studies of organisms where DNA data are inaccessible (*e.g.*, fossils, rare organisms). Wood anatomy has certainly proved useful (Tippo, 1946; Lens *et al.*, 2007; Christenhusz *et al.*, 2010), especially characters linked to cell organization, cell types, or pitting. Here we show that fibre cell wall of reaction tissues, in this case tension wood, may also be useful phylogenetically.

Trees are able to optimize their orientation thanks to the production of asymmetrical maturation stress around the tree. In angiosperms, a special wood with very high tensile stress, called tension wood, is produced on the upper side of the tilted stem. This high tensile stress allows for the active bending of the tree axis (Du & Yamamoto, 2007; Alméras & Fournier, 2009). Tension wood exhibits anatomical differences from normal wood, for example, a lower frequency of vessels (Jourez, Riboux, & Leclercq, 2001; Ruelle *et al.*, 2006). However, the strongest differences are observable at the fibre wall level. The secondary wall of a normal wood cell is composed of three sub-layers (S_1, S_2, S_3) made of cellulose microfibrils oriented at different angles and embedded in a matrix of lignin and hemicelluloses. Whereas S_1 and S_3 are thin, with cellulose microfibrils oriented to nearly 80° compared to the fibre axis, the S_2 layer is much thicker and the angle of microfibrils range from 10 to 20°. In tension wood the cell wall is generally modified by the presence of an inner unlignified layer, the so-called gelatinous layer or G-layer, replacing the S_3 and part of or the entire S_2 layer. It has been recently shown that this layer can be later lignified (Roussel & Clair, 2015). This partially explains why many species were known to produce tension wood lacking G-layers (Onaka, 1949; Fisher & Stevenson, 1981; Clair *et al.*, 2006). Whether lignified or not, tension wood cell wall is homogeneous and characterized by a much lower (up to nil) cellulose microfibril angle compared to normal wood (Chaffey, 2000; Ruelle *et al.*, 2006).

A peculiar fibre wall structure was first discovered in xylem fibres of *Homalium foetidum* Benth., *Homalium luzoniense* Fern.-Vill. and *Olmediella betschleriana* Loes. (Bailey & Kerr, 1935; Daniel & Nilsson, 1996), in which the secondary wall appears multilayered. This peculiar cell wall structure was later demonstrated to occur only in tension wood (Clair *et al.*, 2006; Ruelle *et al.*, 2007a; Fig. 1). Daniel & Nilsson (1996) described this structure in *Homalium foetidum* as a succession of thick layers separated by thin layers with elevated levels of lignin. In *Laetia procera* Eichler, Ruelle *et al.* (2007a) described the thick layers as lightly lignified. Similar cell wall structures were also reported in reaction phloem fibres (Nanko, Saiki, & Harada, 1982; Nakagawa, Yoshinaga, & Takabe, 2012, 2014). In phloem fibres of *Populus* × *canadensis* Moench, Nanko *et al.* (1982) observed that the maximum number of layers was on the upper side of the tilted axis and decreases to normal phloem on the other side. They concluded that the number of layers is related to the intensity of tension wood. Nakagawa *et al.* (2012) observed multi-layered fibres in opposite phloem in *Mallotus japonicus* (L.f.) Müll.Arg. (Euphorbiaceae), but they demonstrated an increase in the number of layers from opposite phloem to reaction phloem. In addition, *Mallotus japonicus* and *Hevea brasiliensis* (Willd. ex A.Juss.) Müll.Arg. (Euphorbiaceae) were reported to form a multilayered secondary wall structure in their tension wood fibres (Encinas & Daniel, 1997; Nakagawa *et al.*, 2012) while *Dipterocarpus* C.F.Gaertn. (Dipterocarpaceae), *Dillenia* L. (Dilleniaceae), *Laurelia* Juss. (Atherospermataceae), and *Elateriospermum* Blume (Euphorbiaceae) formed it in wood fibres, without specifying it was tension wood (Daniel and Nilsson 1996).

The observation of this multi-layered structure raises many questions about its role and the benefits for the plant compared to usual G-layers. Clair *et al.* (2006) showed that tensile stress

measured on *Casearia javitensis* Kunth, a species with multilayered G-layer, is among the highest compared to the other 21 species measured, but they did not show a gap compared to other tension wood types. Ruelle *et al.* (2007b) obtained similar results for *Laetia procera*, compared to 10 species.

Interestingly, this atypical structure is reported in five species of Salicaceae, all belonging to the former Flacourtiaceae: *Homalium luzoniense* and *Olmediella betschleriana* (Bailey & Kerr, 1935), *Homalium foetidum* (Daniel & Nilsson, 1996), *Casearia javitensis* (Clair *et al.*, 2006) and *Laetia procera* (Ruelle *et al.*, 2007a). These results contrast with observations recorded from Salicaceae *s.s.* (*Salix* and *Populus*). Indeed, tension wood has been extensively studied in *Populus*, considered as a model plant for studies of angiosperms (Pilate *et al.*, 2004). These numerous studies report observations of tension wood cell walls with various techniques such as transmission electron microscopy (Araki *et al.*, 1982; Yoshinaga *et al.*, 2012), atomic force microscopy, scanning electron microscopy (Clair & Thibaut, 2001), confocal Raman microscopy (Gierlinger & Schwanninger, 2006), UV or bright field microscopy (Yoshinaga *et al.*, 2012) and phase contrast microscopy (Abedini *et al.*, 2015; Chang *et al.*, 2015). All of these observations describe tension wood cell walls in *Populus* as a single walled G-layer (Fig. 1). *Salix* is also known to have single walled G-layers (Gritsch *et al.*, 2015). In this paper, we will name these single layered G-layers as "usual G-layer" in contrast with "multilayered G-layer" or "multilayered fibres" for G-layers composed of two or more layers (Fig. 1). Both may be lignified or not.

Salicaceae *s.s.*, composed of the genera of *Populus* and *Salix*, has been recently enriched with numerous genera from the former Flacourtiaceae (Chase *et al.*, 2002). The latter family was hard to characterize because it served as a depository, or "garbage bag," for taxa with uncertain affinities (Chase *et al.*, 2002). Several studies have provided molecular and/or morphological data that support the realignment of most of the genera and species to Salicaceae or Achariaceae (Chase *et al.*, 2002; Alford, 2005; Xi *et al.*, 2012), but there is still argument about whether the non-cyanogenic Flacourtiaceae should be treated in a Salicaceae *s.l.* or subdivided even further into Samydaceae, Scyphostegiaceae, and a Salicaceae *sensu medio* (Alford, 2005; Samarakoon, 2015). Alford (2005) argues for the latter because he could find no *morphological* characters that supported Salicaceae *s.l.* while several synapomorphies supported recognition of Samydaceae, Scyphostegiaceae, and Salicaceae *sensu medio*. Given these questions of circumscription as well as the variation in tension wood cell walls, tension wood characters may prove to be a unifying feature or synapomorphy of Salicaceae *s.l.* which was later lost in *Salix*, *Populus*, and their closest relatives.

This study aims to answer how this particular multilayered tension wood is distributed among the species newly classified in the Salicaceae. The topic of this study is two-fold: (1) to clarify the expression of this peculiar tension wood, and (2) to generate new anatomical data for the Salicaceae. Here, we investigate (1) the characteristics of multilayered tension wood and (2) whether the multilayered cell wall is a characteristic of all former Flacourtiaceae newly integrated in Salicaceae. Achariaceae, Goupiaceae, and Lacistemataceae, three families formerly included in or closely linked to the Flacourtiaceae, were also examined for a broader understanding of the distribution of this anatomical character.

Materials and methods

Naturally tilted branches or main axes of Salicaceae were collected in natural forest in four places in French Guiana and Guadeloupe or were provided by the Lyon Botanical Garden

(France), the Nancy Botanical Garden (France), the Strasbourg University Botanical Garden (France), and the experimental unit of Villa-Thuret (INRA, France) (Table 1). Thirty-one species belonging to 14 genera from Salicaceae were studied. The selected genera were drawn from five of the nine tribes of the family, encompassing the major morphological groups. Some of the species are represented by several individuals. Additionally, two species of Achariaceae, one species of Goupiaceae, and three species of Lacistemataceae were added to this study because of their former inclusion in or close relationship to Flacourtiaceae. Finally, because of the surprising results obtained from *Idesia* collected in a botanical garden, a three-year-old tree of *Idesia polycarpa* Maxim. collected at the Kitashirakawa Experimental Station of the Field Science Education and Research Center of Kyoto University in Japan, was artificially tilted to ensure the production of tension wood. The list of the species and their provenances are given in Table 1. Table 2 lists the genera belonging to Salicaceae and highlights the genera observed in the study.

Tension wood was confirmed by the presence of eccentric growth with more wood produced on the upper side of the axis. Sample preparation and observations were performed in Kourou, French Guiana.

3D Laser Scanning Confocal Microscopy

Tension wood samples were observed with a 3D Laser Scanning Confocal Microscope (Keyence VK-9710K). This technique allows for the observation of the topography of the surface of a sample with a resolution of 10 nm. Observations were made on dry blocks after smoothing the surface with a diamond knife on a rotary microtome. This sample preparation produces a nearly perfect surface. However, changes in organization or composition from layer to layer create some topographic traces at the surface of the sample, allowing an easy identification of the cell wall layers or sub-layers.

Optical Microscopy

Wood samples were kept wet until sectioning. Sections 20-50 µm thick were produced with a sliding microtome and stained with Safranin and Alcian blue 8GX in order to observe lignin distribution. Mounted on glass slides, they were observed under bright field with an optical microscope (Olympus BX2, Japan). Unstained sections 2-3 µm thick were produced from some species for UV microscopy in order to validate results obtained via Safranin/Alcian blue staining. These sections were observed under the same microscope but equipped with a Mercury lamp (USH102D USHIO, USA) that generates light filtered with Fluorescence Filter Cubes U-MNU2 (Olympus, Japan, excitation filter: 360–370 nm, dichromatic mirror: 400 nm, emission filter: 420 nm). Lignified cell wall autofluoresce, whereas unlignified layers like the G-layer remain dark (Roussel & Clair, 2015).

Results

Fig. 2 presents tension wood fibres of some of the studied species observed with Laser Scanning Confocal Microscopy (see [Supplementary Material] for other species), and Fig. 3 presents the anatomical sections of seven species stained with Safranin/Alcian blue and observed in bright field with optical microscopy.

A multilayered structure in tension wood cell walls is observable in all former Flacourtiaceae, except in *Idesia polycarpa* and *Idesia polycarpa* var. *vestitata*, and is absent in all studied species from Salicaceae *s.s.* (*Populus* and *Salix*), Achariaceae, Goupiaceae, and Lacistemataceae (Fig. 2). A thick S_2 layer is sometimes present and should not to be confused

with the G-layer, which often stands because it detaches from other layers during sectioning (Clair, Thibaut, & Sugiyama, 2005).

The maximum number of layers in a multilayered wall varies from species to species. Most of the species show only two layers, but can be up to six layers in *Casearia sylvestris* Sw. or *Neoptychocarpus apodanthus* (Kuhlm.) Buchheim (Table 2).
For a given species, the number of layers also varies. In particular, monolayered G-layer, *i.e.* usual G-layers, can be found in the vicinity of multilayered G-layers for some species. In a single tension wood specimen, the number of layers increases progressively from cell to cell from one to multiple layers. Consequently, usual G-layers are hardly found near to multilayered fibres. In a limited number of samples, the multilayered fibres are scarce and hard to find amid the usual G-layers (for instance *Dovyalis caffra* (Hook.f. & Harv.) Warb. from Villa-Thuret Experimental garden, INRA). Whenever multilayered tension wood fibres occur they are often found in intense zones of tension wood, *i.e.* centered in the arc of tension wood.

For all species, the thin interlayers of multilayered tension wood cell wall appear lignified as described by Ruelle *et al.* (2007a). In some of the species, thick layers of multilayered tension wood cell wall appeared lignified with the Safranin/Alcian blue staining (confirmed on unstained samples under UV light) (Fig. 3, Table 2). For instance, all multilayers of *Banara guianensis* Aubl. appear lignified while only some of them were lignified in *Casearia sylvestris*. Multilayers appear unlignified in a few species like *Azara dentata* Ruiz & Pav. and *Carrierea calycina* Franch. (Table 2). Interestingly, thick layers of the tension wood cell wall of *Dovyalis caffra* are unlignified on the sample from Villa-Thuret Experimental garden (INRA) but partly lignified on the sample from the Strasbourg University Botanical Garden. Similarly, the thick layer of tension wood cell wall of *Laetia procera* looks partially lignified in our sampling, whereas Ruelle *et al.* (2007a) found these layers unlignified with Safranin/Alcian blue staining, although slightly lignified with Wiesner reaction.

Discussion

Tension wood. Despite the scarcity of previous observations in the literature (Ruelle *et al.*, 2007a), we confirm that the multilayered wall occurs in the tension wood fibres of a large number of genera and species (11 genera and 21 species). When multilayered cell walls are formed, they are always observed on the upper side of the tilted axis, *i.e.* only in tension wood. We did not observe multilayered cell walls in axes without tension wood even when the given species is able to form multilayered wall cells. Thus, the position of sampling in the plant body for observing the presence or absence of such multilayered cells is critical. In this study, we collected only axes highly susceptible to have formed tension wood during past radial growth (tilted branches or bent main axis). So the present investigations give a clear idea of the distribution of the multilayered cells in tension wood of these species of Salicaceae.

Variability in the number of layers and in the distribution of multilayered wall in tension wood.

The number of layers of the tension wood cell wall varies between species, but also within a species or within a single individual. Within a sample, tension wood with the usual G-layer can occur near multilayered fibres. Similar observations was made by Daniel & Nilsson (1996), although the authors did not identify this peculiar structure to be a characteristic of tension wood. At the species level it is interesting to note that the tilted branch of *Laetia procera* showed here a variation from 1–5 layers, whereas 4–8 layers were observed by Ruelle et al. (2007*b*) on naturally tilted trees. It is suspected that the number of layers of one species would be linked to

the intensity of reaction wood formation, as it has been shown for reaction phloem fibres (Nanko *et al.*, 1982). It also seems that some species may have a higher maximal number of layers. Nevertheless, those conclusions cannot be reached at this point and would require a more complete study with artificially tilted stems grown in a controlled environment. It would also be interesting to investigate if the formation of the usual G-layer or multilayered G-layer depends on the mechanical stimulus. For example, in *Dovyalis caffra* from Villa-Thuret Experimental garden (INRA), most fibres formed a typical G-layer and very few multilayered fibres occurred. One can wonder whether the degree of mechanical stimulus has an effect on the resulting fibre wall structure in some species, and this would have a bearing on the distinction between the former Flacourtiaceae and Salicaceae *s.s.*

Tension wood and lignification. In *Homalium foetidum* (Daniel & Nilsson, 1996), the thick layers show weak lignification whereas in *Laetia procera* (Ruelle *et al.*, 2007a), some multilayered fibres are even more lignified than other multilayered fibres. The role of lignification in tension wood is not fully understood yet and is still being investigated (Roussel & Clair, 2015). It is, for instance, not clear whether lignification would occur in some species and not in others or whether it is a result of a peculiar environment or mechanical stimulus. Does *Dovyalis caffra* lignify or not in reaction to the environment or, for instance, due to the plant ontogeny (Roussel & Clair, 2015) or some other unknown factor ? It can nonetheless be noted that we did not observe an obvious pattern of lignification at the level of a species or a genus. A better understanding of the triggers and the role of lignification in tension wood will be necessary to further interpret our observations.

Tension wood fibre wall structure and phylogeny.
Although our sample does not include all genera and species of Salicaceae, our results give a clearer representation of the family on the basis of this anatomical character. Chase *et al.* (2002) proposed nine tribes constituting the family (Table 3), primarily following Lemke (1988). We studied 14 genera in five tribes. The seven species studied within tribe Saliceae (*Populus* and *Salix*) did not show multilayered walls. Conversely, among the 12 genera and 24 species studied from the four other tribes, all species except *Idesia polycarpa* exhibited multilayered tension wood fibre walls. Thereby, among the genera observed in this study (representing 27% of the genera in this last group of tribes), nearly all of them showed multilayered tension wood cell walls, supporting the idea that this anatomical character would also be present in most of the other non-studied genera.

Nevertheless, the particular exception is *Idesia polycarpa*, a species for which we could not find multilayered tension wood even within a zone of severe tension wood produced in an artificially tilted tree. *Idesia polycarpa* is therefore probably unable to form multilayered tension wood cell walls like both *Salix* and *Populus*. Indeed, long ago Hallier (1908, 1912) suggested that *Idesia polycarpa* is closely connected to Salicaceae, an idea relayed by Miller (1975) and supported by the phylogenetic analysis of morphological and DNA data (Leskinen & Alström-Rapaport, 1999; Alford, 2005). Miller (1975) previously suggested a close relationship between Salicaceae and some Flacourtiaceae and attested to the close relationships in wood anatomy between *Idesia*, *Itoa*, *Salix*, and *Populus*.
Chase *et al.* (2002) clearly show the close relationships between *Idesia*, *Benettiodendron* Merr., *Itoa* Hemsl, *Poliothyrsis* and *Salix* and *Populus*, and Alford (2005) added *Carrierea* Franch., *Macrohasseltia* L.O.Williams, and *Olmediella* Baill. to this clade (Fig. 4). In this clade, only *Poliothyrsis sinensis*, *Carrierea calycina*, and *Olmediella betschleriana* (Bailey & Kerr, 1935) produced multilayered tension wood cell walls. It would therefore be of interest to investigate

the tension wood of *Bennettiodendron* spp., *Macrohasseltia macroterantha*, and *Itoa orientalis* since no information about their tension wood is currently available. Due to the particular position of *Idesia polycarpa* and the limited phylogenetic resolution in this clade, the hypothesis is proposed that *Idesia polycarpa* and both *Salix* and *Populus* may have a recent common ancestor which lost the ability to form multilayered tension wood.

Therefore, on the basis of the multilayered tension wood cell wall, Salicaceae appear structured in two parts: the Salicaceae *s.s.* without multilayered tension wood fibre walls and the former Flacourtiaceae, with *Idesia polycarpa* (and maybe *Bennettiodendron*, *Macrohasseltia macroterantha*, and *Itoa orientalis*) positioned in between. The results clearly distinguish a core ex-Flacourtiaceae on the basis of tension wood. Tension wood is therefore an additional character, together with morphological and genetic characters, useful for understanding the evolution of Salicaceae.

Whilst this anatomical feature was almost systematically present in the former Flacourtiaceae newly classed in Salicaceae *s.l.*, it appears that several species of Euphorbiaceae, a family in the same order of angiosperms (Xi *et al.*, 2012), developed it as well (Daniel & Nilsson, 1996; Encinas & Daniel, 1997; Nakagawa *et al.*, 2012; unpublished results). Additional sampling there, too, may provide broader insights to the evolutionary significance of this character.

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Fig. 1. Comparison of normal wood (A, C, E, G) and tension wood (B, D, F, H) fibre wall in *Populus* (A, B, C, D) and *Laetia procera*. (E, F, G, H) observed in bright field after staining with Safranin/Alcian blue (A, B, E, F) and with 3D Laser Scanning Confocal Microscope (C, D, G, H). *Populus* tension wood is characterised by a typical unlignified G-layer and *Laetia* tension wood by a multi-layered G-layer. Arrows indicate the G-layer and/or its thickness (g) and an artefact of residual traces of the diamond knife (k).

Fig. 2. Transverse sections of tension wood fibres observed with 3D Laser Scanning Confocal Microscope . Scale bar : 10µm. A-B: Achariaceae, C: Goupiaceae, D: Lacistemataceae, E-R: Salicaceae *s.l.* A: *Carpotroche* sp., B: *Kiggelaria africana*, C: *Goupia* sp., D: *Lacistema aggregatum*, E: *Idesia polycarpa*, F: *Populus alba* (thick S_2 layer), G: *Salix lucida*, H: *Azara dentata*, I: *Banara guianensis*, J: *Carrierea calycina*, K: *Casearia* cf. *decandra*, L: *Dovyalis caffra*, M: *Homalium guianense*, N: *Laetia procera*, O: *Neoptychocarpus apodanthus*, P: *Poliothyrsis sinensis*, Q: *Ryania speciosa*, R: *Xylosma benthamii*. A-G: One layer observed in the G-layer, H-R: More than two layers observed in the G-layer.

Fig. 3. Transverse sections of opposite wood (A, C, E, G, I, K) and tension wood (B, D, F, H, J, L) stained with Safranin/Alcian blue. Scale bar: 20 µm. A-B: Achariaceae, C-L: Salicaceae *s.l.*. (A, B) *Carpotroche* sp., (C, D) *Banara guianensis*, (E, F) *Carrierea calycina*, (G, H) *Idesia polycarpa* var.*vestitata*, (I, J) *Laetia procera*, (K, L) *Salix lucida*. (H,L): One layer observed in the G-layer, (B,D,F,J): More than two layers observed in the G-layer. Note the presence of lignin in the G-layer.

Fig. 4. Schematic representation of phylogenetic relationships in Salicaceae *s.l.*, based on Chase *et al.* (2002) and Alford (2005). Arrow shows immediate relatives of *Salix* and *Populus* according to Alford (2005). Studied species and species from the literature are written in black. Species with multilayered tension wood are underlined.

Table 1. Species of trees used in this study. Samples were collected in Europe (France), the Caribbean (Guadeloupe), South America (French Guiana) and Asie (Japan). Location of sampling performed in French Guiana are presented as: Place name, nearest city, FG. N = number of trees collected, V= number of vouchers collected, FG = French Guiana, JP = Japan, FSERC = Field Science Education and Research Center.

Genera	Species	Family	N	V	Sampling location
Carpotroche	sp.	Achariaceae	3	3	Paracou, Sinamary, FG
Kiggelaria	*africana*	Achariaceae	2		Lyon botanical garden Villa-Thuret Experimental garden (INRA), Antibes
Goupia	sp.	Goupiaceae	1		Montagne des singes, Kourou, FG

Lacistema	*aggregatum*	Lacistemataceae	1	1	Paracou, Sinamary, FG
Lacistema	*pubescens*	Lacistemataceae	1	1	Paracou, Sinamary, FG
Lacistema	sp.	Lacistemataceae	1	1	Montagne des singes, Kourou, FG
Azara	*dentata*	Former Flacourtiaceae	1		Lyon Botanical Garden
Banara	*guianensis*	Former Flacourtiaceae	1		Montagne des singes, Kourou, FG
Carrierea	*calycina*	Former Flacourtiaceae	1		Strasbourg University Botanical Garden
Casearia	cf *decandra*	Former Flacourtiaceae	3	3	Paracou, Sinamary, FG
Casearia	*commersoniana*	Former Flacourtiaceae	1	1	Paracou, Sinamary, FG
Casearia	*grandiflora*	Former Flacourtiaceae	1	1	Piste Paul Isnard, Saint-Laurent, FG
Casearia	*guianensis*	Former Flacourtiaceae	2	2	Crique Passoura, Kourou, FG
Casearia	*javitensis*	Former Flacourtiaceae	2	2	Crique Passoura, Kourou, FG Montagne des singes, Kourou, FG
Casearia	*pitumba*	Former Flacourtiaceae	3	3	Montagne des singes, Kourou, FG
Casearia	*sylvestris*	Former Flacourtiaceae	3	3	Paracou, Sinamary, FG
Dovyalis	*caffra*	Former Flacourtiaceae	2		Strasbourg University Botanical Garden Villa-Thuret Experimental garden (INRA), Antibes
Homalium	*guianense*	Former Flacourtiaceae	1	1	Crique Passoura, Kourou, FG
Homalium	*racemosum*	Former Flacourtiaceae	1		Guadeloupe
Idesia	*polycarpa* *polycarpa* var.*vestitata*	Former Flacourtiaceae	2		Kyoto University FSERC(JP) Strasbourg University Botanical Garden
Laetia	*procera*	Former Flacourtiaceae	1	1	Montagne des singes, Kourou, FG
Neoptychocarpus	*apodanthus*	Former Flacourtiaceae	1	1	Paracou, Sinamary, FG
Poliothyrsis	*sinensis*	Former Flacourtiaceae	2		Strasbourg University Botanical Garden Villa-Thuret Experimental garden (INRA), Antibes
Ryania	*speciosa* *speciosa* var.*bicolor*	Former Flacourtiaceae	2		Paracou, Sinamary, FG Montagne des singes, Kourou, FG
Xylosma	*benthamii*	Former Flacourtiaceae	1		Paracou, Sinamary, FG
Xylosma	*congesta*	Former Flacourtiaceae	1		Villa-Thuret Experimental garden (INRA), Antibes
Xylosma	*flexuosa*	Former Flacourtiaceae	1		Strasbourg University Botanical Garden
Xylosma	*japonica*	Former Flacourtiaceae	1		Strasbourg University Botanical Garden
Populus	*alba*	Salicaceae s.s.	1		Nancy Botanical Garden
Populus	*nigra (italica)*	Salicaceae s.s.	1		Nancy Botanical Garden
Populus	*trichocarpa*	Salicaceae s.s.	1		Nancy Botanical Garden
Populus	*deltoides* × *nigra*	Salicaceae s.s.	1		Nancy Botanical Garden
Salix	*lucida*	Salicaceae s.s.	1		Nancy Botanical Garden
Salix	*myrsinifolia*	Salicaceae s.s.	1		Nancy Botanical Garden
Salix	*purpurea*	Salicaceae s.s.	1		Nancy Botanical Garden

Table 2. Genera studied among Salicaceae, based on Chase *et al.* (2002). The column 'Described species' indicates the number of species observed in a given genus. Tension wood cell wall, the number of layers min-max of the G-layer and the presence of lignification of the G-layer are described. (*) This genus has now been moved to a distantly related family (Alford, 2006).

Sub family	**Tribes**	**Genera**	**Described species**	**Number of layers**	**Tension wood lignification**
Incertae sedis		*Oncoba*			
Salicoideae	*Abatieae*	*Abatia* *Aphaerema*			
	Bembicieae	*Bembicia*			
	Flacourtieae	*Azara* *Bennettiodendron*	1	1-3	Unlignified
		Carrierea	1	1-2	Unlignified
		Dovyalis	1	1-3	Partly lignified or unlignified
		Flacourtia *Idesia* *Itoa* *Lasiochlamys* *Ludia* *Olmediella*	1	1	Unlignified
		Poliothyrsis *Priamosia* *Tisonia*	1	1–3	Unlignified
		Xylosma	4	1-3 (*flexuosa*) 2-3 (*japonica*) 1-4 (*benthamii*, *congesta*)	Partly lignified

	Homalieae	*Bartholomaea* *Bivinia* *Byrsanthus* *Calantica* *Dissomeria* *Gerrardina* (*)			
		Homalium	2	1-3 (*guianense*) 1-4 (*racemosum*)	Partly lignified
		Neopringlea *Trimeria*			
	Prockieae	*Banara* *Hasseltia* *Hasseltiopsis* *Macrohasseltia* *Neosprucea* *Pineda* *Pleuranthodendron* *Prockia*	1	2-3	Lignified
	Saliceae	*Populus*	4	1	Unlignified
		Salix	3	1	Partly lignified or unlignified
	Scolopieae	*Hemiscolopia* *Mocquerysia* *Phyllobotryon* *Phylloclinium* *Pseudoscolopia* *Scolopia*			
Samydoideae	*Samydeae*	*Casearia*	8	1-3 (*grandiflora*, *guianensis*, *javitensis*, *pitumba*) 1-4 (*cf decandra*,*javitensis*, *sylvestris*) 2-4 (*cf decandra*, *pitumba*) 1-5 (*commersoniana*, *sylvestris*) 1-6 (*sylvestris*)	Lignified or partly lignified
		Euceraea *Hecatostemon* *Laetia* *Lunania*	1	1-5	Partly lignified
		Neoptychocarpus *Ophiobotrys* *Osmelia* *Pseudosmelia*	1	1-6	Lignified
		Ryania *Samyda* *Tetrathylacium* *Zuelania*	2	1-3	Partly lignified
Scyphostegioideae		*Scyphostegia*			

Table 3. Number and percentage of the studied genera among Salicaceae and of the presence of a multilayered wall in tension wood fibres of the studied genera.

Tribe (or genus)	**Genera**	**Studied genera**		**Studied genera with multilayered wall**	
	Number	Number	%	Number	%
(*Oncoba*)	1	0	**0**	–	–
Flacourtieae	14	6	**42**	5 (- *Idesia*)	**83**
Homalieae	9	1	**11**	1	**100**
Saliceae	2	2	**100**	0	**0**
Scolopieae	6	0	**0**	–	–
Abatieae	2	0	**0**	–	–
Bembicieae	1	0	**0**	–	–
Prockieae	8	1	**12**	1	**100**
Samydeae	13	4	**30**	4	**100**
(*Scyphostegia)*	1	0	**0**	–	–
	57	14	**24**	11	**78**

Supplementary material 1. Transverse sections of tension wood fibres observed with 3D Laser Scanning Confocal Microscope . Scale bar : 10µm. A-B: Lacistemataceae, C-S: Salicaceae *s.l.* A: *Lacistema pubescens*, B: *L.* sp (thick S_2 layer), C: *Idesia polycarpa* var. *vestitata*, D: *Populus nigra (italica)*, E: *P. trichocarpa*, F: *P. deltoides* × *nigra*

(thick S_2 layer), G: *Salix myrsinifolia*, H: *S. purpurea* (thick S_2 layer), I: *Casearia commersoniana*, J: *C. grandiflora*, K: *C. guianensis*, L: *C. javitensis*, M: *C. pitumba*, N: *C. sylvestris*, *O*: *Homalium racemosum*, *P*: *Ryania speciosa* var. *bicolor*, Q: *Xylosma congesta*, , R: *X. flexuosa*, S: *X. japonica*. A-H: One layer observed in the G-layer, I-R: More than two layers observed in the G-layer.